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Evaluation of Shubhodoya Mycorrhizal Bio-fertilizer for Enhancing Rooting of Nursery Tea Plants.

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ABSTRACT

Bio-fertilizers have not been exploited in Kenya on tea production despite benefits demonstrated in many crops. The bio-fertilizers are organisms that enrich nutrient soil quality. Plants have beneficial relationships with such organisms. Shubhodoya mycorrhizal bio-fertilizer is a consortium of three different species of *Glomus* mycorrhizal fungus, produced in laboratory under sterile conditions. They are cultured and used for inoculating seed or soil or both under ideal conditions to increase availability of plant nutrients. A nursery experiment was conducted to evaluate the efficacy of Shubhodaya mycorrhizal bio-fertilizer (SMB) in enhancing growth of two tea clones, TRFK 306 and EPK TN14-3. Different rates of SMB (0.6g, 0.9g, and 1.2g), standard treatment (6g diammonium phosphate) and control (no fertilizer) were laid out in randomized complete block design. Sleeved seedlings with the soil were randomly sampled from the nursery for analysis of soil pH, assessment of growth parameters and mycorrhizal colonization were conducted. Root samples were used to determine dry weight and to conduct assays for VAM infection. The SMB did not have adverse effects on soil pH in the nursery. Plants subjected to the DAP treatment all died after weeks 27. Increasing application of SMB rates and frequency increased shoot growth. SMB at 1.2 g exhibited the lowest shoots dry weight while SMB at 0.6 and 0.9g had the highest. After 62 weeks from planting, there was an abrupt increase in shoot dry weight. Successful colonization of tea roots with inoculation of SMB was also observed. SMB colonized the tea roots an indication that it has potential for exploitation.

INTRODUCTION

Mycorrhiza is a symbiotic relationship between arbuscular mycorrhizal fungi and roots of majority of vascular plants. Mycorrhizal fungi are species of fungi that intimately associate with plant roots forming a symbiotic relationship, with the plant providing sugars for the fungi and the fungi providing nutrients such as phosphorus, to the plants (*1*). Mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and release to plant cells in root tissue (*1*). A mycorrhiza ("fungus–root") is a type of endophitic, biotrophic, mutualistic symbiosis prevalent in many cultivated and natural ecosystems. Mycorrhiza plays an important role on enhancing plant growth and yield due to an increase supply of phosphorus and other lacking nutrients to the host plant (*2*). Mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non–mycorrhizal plants (*3*). Plants inoculated with endomycorrhiza were be more resistant to some root diseases (*4*). Mycorrhiza increase root surface area for water and nutrients uptake. The use of mycorrhizal bio-fertilizer helps to improve branching of plant roots, and the mycorrhizal hyphae grow from the root to soil enabling the plant roots to have contact with wider area of soil surface, hence, increasing the absorbing area for water and nutrients of the plant root system (*5*). Therefore, plants with mycorrhizal association have high efficiency for nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, zinc, and copper) absorption and increased resistance to drought (*6*). The fungi withdraw glucose from plant roots and act as a sink for carbohydrates (*5*). arbuscular mycorrhizal fungi (AMF) are important in ecological agriculture because of the benefits they provide to majority of cultivars and conservation of the environment by acting as bio-fertilizers, bio-protectors and bio-control agents (*2*). In the framework of sustainable agriculture, the soil is considered as an active element of the system, composed of interrelated physical, chemical and biological factors, the AMF forming part of a biological microcosm (*1*). Several carrier based mycorrhizal inoculants are available. However, their quality varies. Several efforts have been made in the past to supply quality inoculants. Recently, root organ cultures of the AMF have

been developed. Shubhodoya Mycorrhizal Bio-fertilizer (SMB) is one such commercial carrier based mycorrhizal bio-fertilizer. The product is a combination of three different highly adaptable vesicular arbuscular mycorrhiza (VAM) species of *Glomus* mycorrhizal fungus. The product contains 100,000 infective propagules of VAM per kilogram product. The objectives of this study were to determine the ability of SMB to improve and enhance rooting of TRFK 306 and EPK TN14-3 seedlings in a nursery and to evaluate the effects of SMB on growth of nursery tea plants under Kericho conditions.

MATERIALS AND METHODS

Site description and experimental set up

A nursery experiment was conducted at Tea Research Institute (Kenya), Timbilil Estate, Kericho. The site is at an altitude of 2178m above Mean sea level, latitude 00 22' S and longitude 350 21' E.

Design and treatments

The experiment in tea nursery was laid out in split-plot design in randomized complete block layout with five treatments rates split between two clones and replicated three times. The trial used forest soil in sleeves with 3 rates of SMB (0.6, 0.9 and 1.2g), 0.6g of DAP and a control as main treatments and two cultivars (EPK TN14-3 and TRFK 306) as sub-treatments. Treatments were applied by thoroughly mixing the requisite amount of SMB powder with the sleeve soil. Each sub-treatment consisted of 200 polythene sleeves replicated three times. A 1000 cuttings per clone were planted. A single node cutting was planted into each sleeve. Cuttings were raised as per the recommended nursery practices (*7*).

Nursery sampling and analyses

To determine the effect of the product on the acidity of the soils, soil samples for baseline pH, subsequent sampling were collected randomly from the sleeves on the bed, transferred to clear polythene bags and labelled for pH determination in the laboratory. Twenty nursery destructive sampling were done for pH, roots length, colonization, fresh and dry shoots and root weight determination. The first destructive sampling was done on 31st July 2014 and the subsequent sampling after every month. During first and second destructive sampling shoots were also analyzed for nutrients content.

After determination of pH (1:1 soil:water), the samples were spread in a drying shade before sieving through a 2mm sieve. The samples were then analyzed for extractable phosphorus (dilute HCl-H_2SO_4), extractable potassium, calcium, magnesium and manganese (1M Ammonium nitrate) using standard methods (*8*).

For the plant samples, the root and shoot were separated and fresh weight recorded. These were then oven-dried at temperature of 105^0C for 24 hours before weighing and milling. Total nitrogen, phosphorus, potassium, calcium, magnesium and manganese were analyzed using standard methods (*8*).

Fungal association in roots

To assess the VAM infection level, root samples collected from the nursery trial were carefully washed and cut into 1cm length. The cut pieces were cleared using 10% potassium hydroxide solution then stained in 1% trypan blue-lacto phenol for 12hrs. The pieces were placed on the glass slide for microscopic observation at magnification of X400. The percent number of roots infected with either vesicles, arbuscular or hyphae was then estimated using Equation 1.

$$\frac{\text{Number of root pieces infected} \times 100}{\text{Total number of roots}} \quad \text{..... Equation 1}$$

Statistical Analysis

The data were subjected to the analysis of variance (ANOVA) using the MSTAT C software package (*9*). Means were separated least significant difference (LSD) method at pd"0.05.

RESULTS AND DISCUSSION

Nursery soil pH

The pH of the soil under SMB treatments reduced considerably from a high of 4.44 to a low of 3.86 under clone TRFK 306. Similarly the pH under DAP treatment reduced from 4.16 to 3.95 under EPK TN 14-3. The pH changes are comparable to the changes in the pH under control (Table 2). This could be attributed to leaching of bases out of sleeves during watering and not the treatments. The observed changes were expected since soil pH is influenced by many soil chemical parameters and may change seasonally depending on weather and the external inputs used (*10*). Therefore, SMB did not have adverse effects on soil pH. Despite the reduction, the pH remained within suitable range for nursery use (*7*).

Root sampling

The first sampling was done at 4 weeks after planting and followed with three more samplings at 9, 14 and 18

Table 2: Effect of SMB on nursery soil pH

Sampling time	Clone	0g	6g DAP	0.6g SMB	0.9g SMB	1.2g SMB	Mean
At planting	TRFK 306	4.10	4.02	4.44	4.10	4.12	4.16
	EPK TN14-3	4.15	4.16	4.58	4.14	4.22	4.25
End of trial	TRFK 306	3.81	3.93	3.86	3.82	3.71	3.83
	EPK TN14-3	3.84	3.95	3.85	3.82	3.68	3.83

weeks respectively. Plants were observed for presence or absence of roots (Table 3).

At 4 and 9 weeks, none of the plants had developed any root. At weeks, 14 and 18 all treatments except DAP had developed roots. However, there was no difference between the treatments and clones.

Roots dry weight

Feeder root weight was determined by measuring total root dry weight over time (Figure 1). Generally, roots dry weight increased gradually over time. At 78 weeks after planting, there was a sudden increase in root dry weight. All other treatments were similar but varied significantly from DAP treatment which had poor root development (Table 4). There was no significant difference between clones. The findings indicate that the root development did not benefit from application of SMB. The use of mycorrhizal bio-fertilizer has been reported to help improve higher branching of plant roots (*5*). This is in contrast with the findings of this study, in which, application of SMB at the rates and frequency used in this study did not increase root growth.

Shoots dry weight

Plants subjected to the DAP treatment all dried up after weeks 27. In the other plots, shoots dry weight increased over time. SMB at 1.2 g exhibited the lowest shoots dry weight while SMB at 0.6 and 0.9g had the highest. At 62 weeks after planting, there was an abrupt increase in shoot dry weight (Figure 2, Table 5). Better growth of seedlings due to mycorrhiza inoculation have been reported chilli (*11*), 1982) and tea (*12*). These observations concur with the findings of this study at SMB rates of 0.6 and 0.9g and suggest that SMB may increase shoot growth even in nursery tea plants.

Analysis of variance of the mean of shoots dry weight of the same period indicate all other treatments were similar but varied significantly from DAP treatment (Table 5).

Mycorrhizal growth and development

Mycorrhizal development was first observed at week nine after planting. However, there was no mycorrhizal colonization of roots up to the 22nd week after planting. The means of the mycorrhizal structures (hyphae, vesicles

Table 3: Root growth for the first four sampling

Clone/ Weeks after planting	0g			6g DAP			0.6g SMB			0.9g SMB			1.2g SMB		
	9	14	18	9	14	18	9	14	18	9	14	18	9	14	18
TRFK 306	0	1/3	2/3	0	0/3	0/3	0	3/3	3/3	0	2/3	2/3	0	0/3	3/3
EPK TN14-3	0	0/3	3/3	0	1/3	2/3	0	1/3	3/3	0	1/3	2/3	0	1/3	3/3

*The numerator refers to the number with roots while the denominator refer to total sampled plants

Table 4: Effect of clone and treatment on mean roots dry weights between 22 and 82 weeks after planting

	Treatments					Means
Clone	0 g	6 g DAP	0.6g SMB	0.9 g SMB	1.2 g SMB	
TRFK 306	1.018	0	1.154	0.997	0.965	0.827
EPK TN14-3	2.169	0	1.741	1.945	2.067	1.584
Treatment Means	1.593	0	1.448	1.471	1.516	
CV (%)			93.44			227.48
LSD, (p=0.05)			0.634			NS

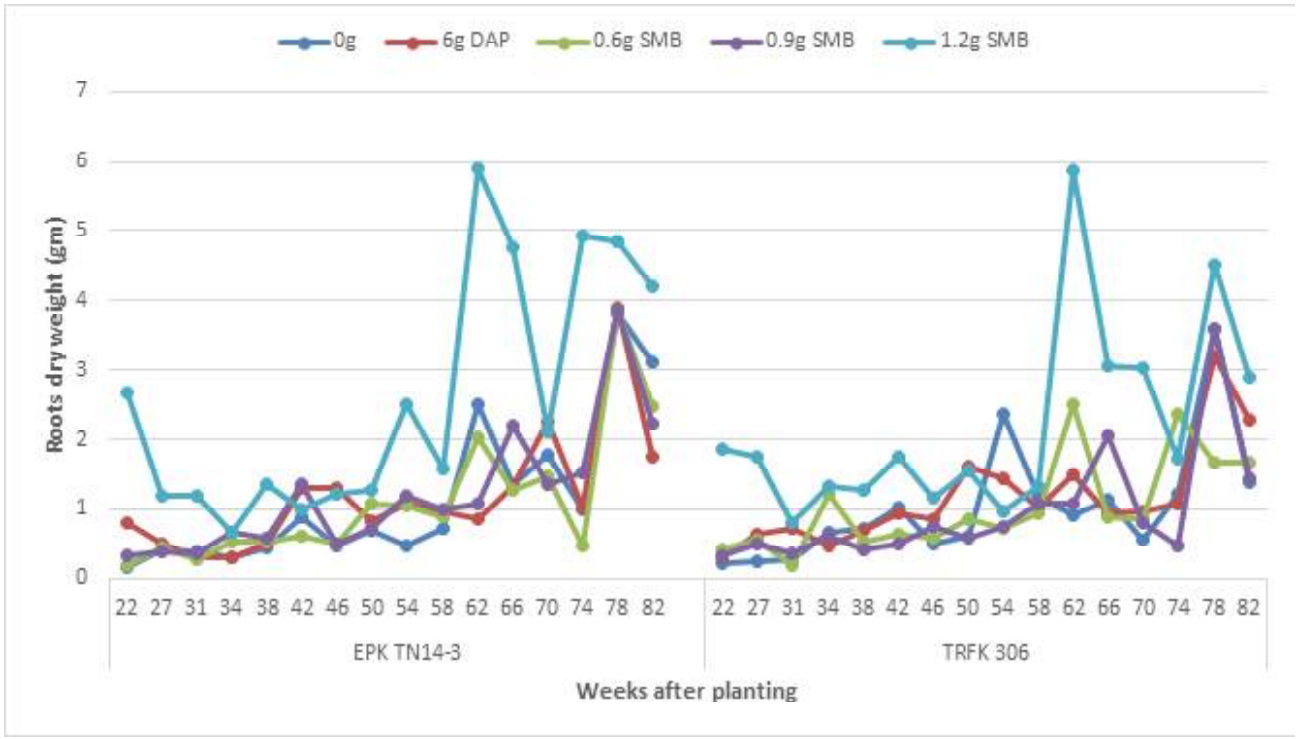


Fig. 1: Effects of treatments on roots dry weight over time

and arbuscules) up to 70 weeks after planting are shown in Table 6. Clone TRFK 306 had higher (pd"0.05) number of hyphae (1.34) compared to EPK TN 14-3. The DAP and control treatments has the lowest (pd"0.05) number of hyphae, compared to others treatment, which had similar values. The interactions between the SMB and clone were significant (pd"0;05), suggesting that response to the treatments varied with clones. Clone TRFK 306 had more

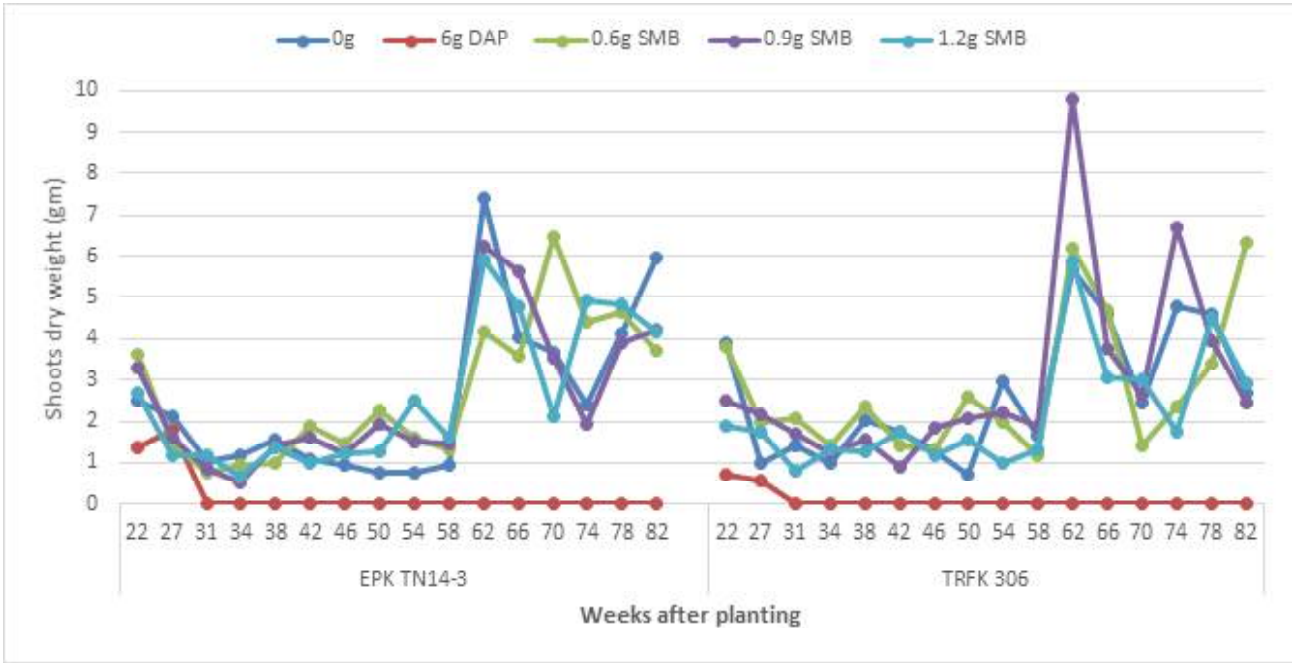


Fig. 2: Effects of clones and treatments on shoots dry weight over time

Table 5: Effect of clones and treatments on mean shoots dry weights between 22 and 82 weeks after planting

Clone	Treatments					Means
	0 g	6 g DAP	0.6g SMB	0.9 g SMB	1.2 g SMB	
TRFK 306	2.649	0.082	2.752	3.006	2.142	2.126
TN 14/3	2.532	0.198	2.702	2.556	2.591	2.116
Treatment Means	2.591	0.14	2.727	2.781	2.367	2.121
CV (%)			51.55			37.21
LSD, p=0.05			0.62			NS

(pd"0.05) vesicles than EPK TN 14-3. However, the number of vesicles in the SMB treatments was not significantly different from the nil control. The mycorrhizal arbuscules development also varied (pd"0.05) with clones. Clone TRFK 306 had the higher (pd"0.05) number of arbuscules than EPK TN 14-3. SMB at 1.2g had significantly the highest number of arbuscules than all other trends.

From the 70th to 82nd week, there was no significant difference between the clones in number of all mycorrhizal structures. However, SMB at 1.2g had significantly the

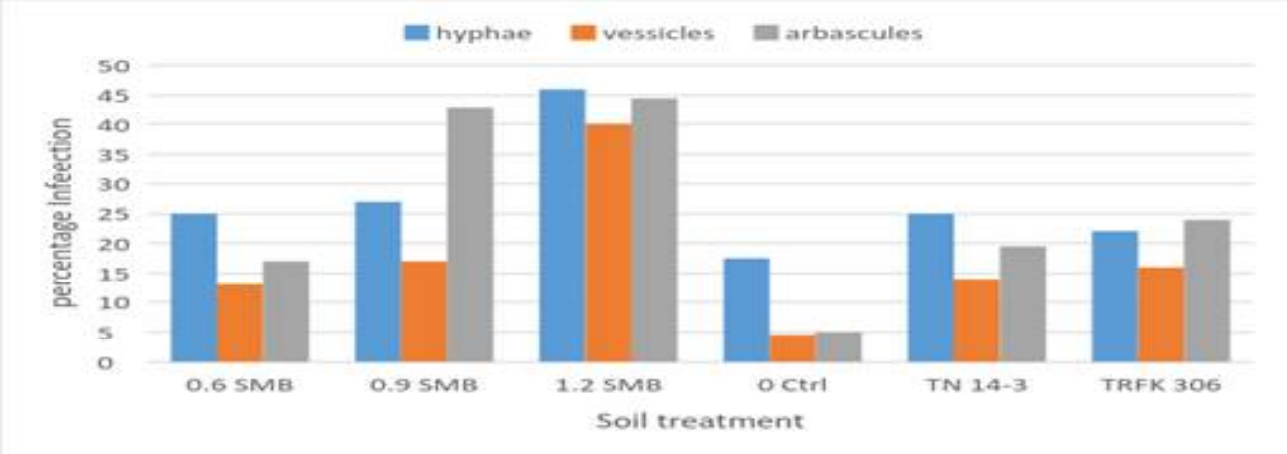
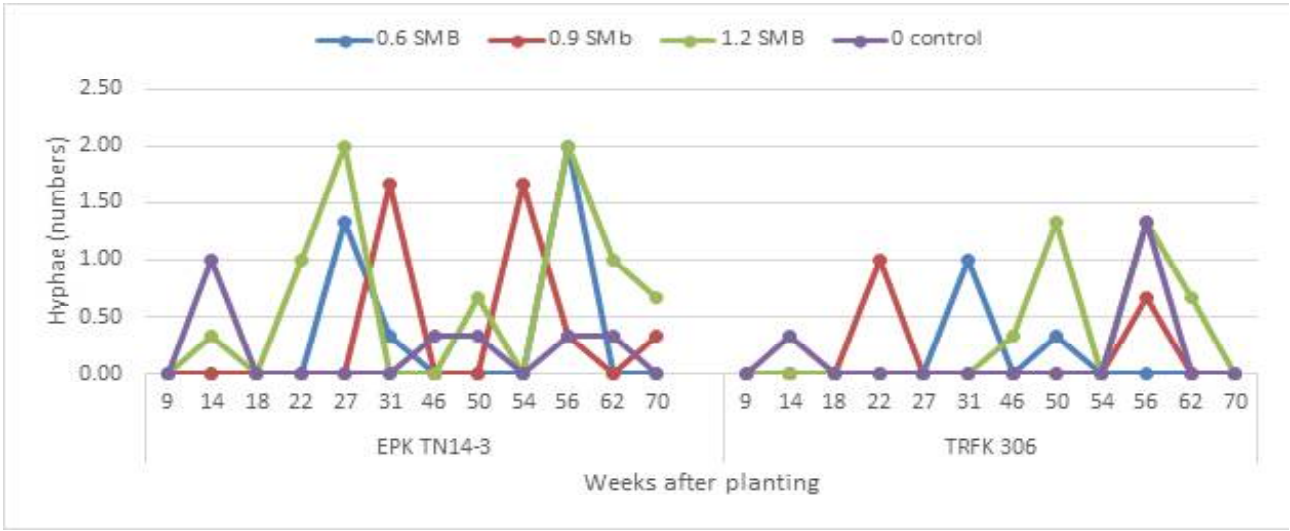


Fig. 3: Colonization of different clones by mycorrhizal hyphae, vesicles and arbuscular subjected to different SMB rates in the nursery test. (70 to 82 week)

Table 6: Growth of Shubhodaya Mycorrhizal hyphae (in number of pieces out of ten) when subject to different SMB rates (9th to 70th week)

Treatment	Clones		Mean treatments	Clones		Mean treatments	Clones		Mean treatments
	Mycorrhizal hyphae			*Mycorrhizal vesicles*			*Mycorrhizal arbuscules*		
	EPK TN14-3	TRFK 306		EPK TN14-3	TRFK 306		EPK TN14-3	TRFK 306	
0.6 SMB	1.0(0.82)	6.3(1.98)	1.4a	1.0 (0.69)	3.7 (1.50)	1.09ab	1.3 (0.92)	3.7 (1.5)	1.21b
0.9 SMB	3.0(1.36)	2.3(1.2)	1.3ab	2.3 (1.13)	3.7 (1.42)	1.28a	1.7 (1.00)	4.0 (1.52)	1.26b
1.2 SMB	2.0(1.06)	8.(2.14)	1.6a	2.3 (1.11)	3.7 (1.48)	1.3a	3.7 (1.48)	7.7 (1.99)	1.73a
6g DAP	1.0(0.69)	1.0(0.69)	0.7c	1.0 (0.69)	1.0 (0.69)	0.69b	1.3 (0.92)	1.0 (0.69)	0.81b
0	3.0(1.33)	1.0(0.69)	1.0c	1.3 (0.83)	1.3 (0.92)	0.88ab	1.7 (0.92)	2.3 (1.06)	0.99b
Mean	1.05b	1.34a		0.98b	1.20a		1.05b	1.35a	
C.V. (%	23.05			38.8			31.7		

Figures in parenthesis are Loge (x+1) transformation of mycorrhizae scores



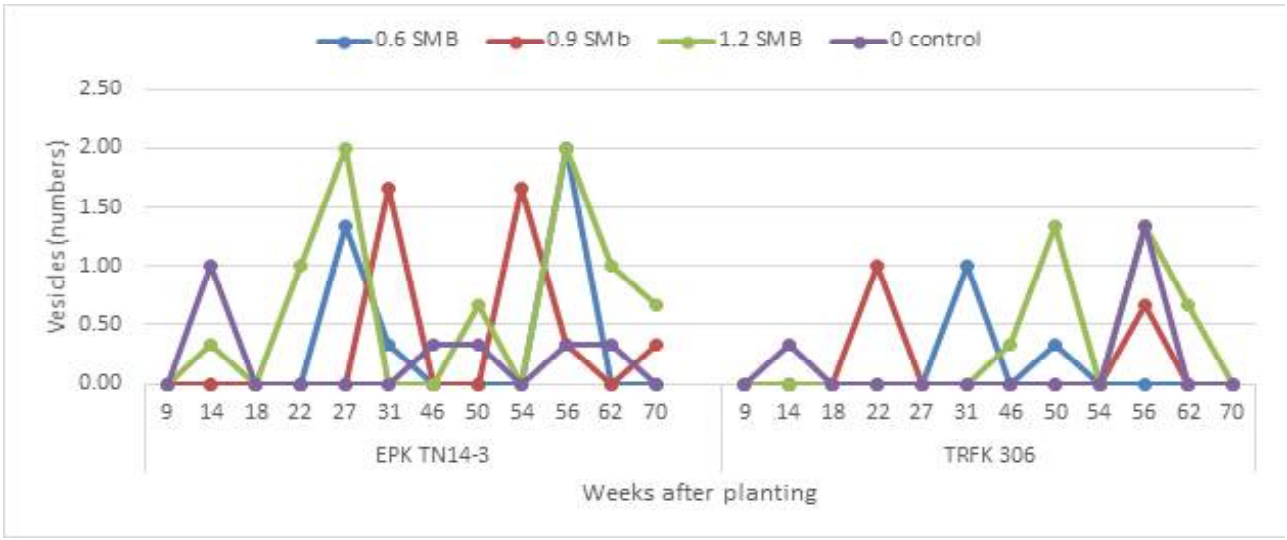


Figure 4a, b, and c: Effect of clones and SMB on mycorrhizal structures

higher number of hyphae and vesicles than all the other treatments. The number of arbuscules was higher at 0.9 and 1.2g SMB (Figure 3).

The means of the mycorrhizal structures (hyphae, vesicles and arbuscules) from the period 9 to 70 weeks after planting are shown in Figure 4a, b, c. There was a general increase in number of hyphae and vesicles between week 9 and 70 but arbuscules show no trend at all. In a previous study on inoculated roots of tea plants (*12*), significant sufficient colonization had been observed. The results concur with observations from this study and suggest successful colonization of tea roots with inoculation of SMB. This implies that use of SMB at appropriate rates would be beneficial in nursery tea plants

In conclusion, the SMB treatments did not have adverse effects on soil pH in the nursery. Application of SMB at the rates and frequency used in this study did not increase root growth but increased shoot growth in nursery plants. Successful colonization of tea roots with inoculation of SMB was observed. The SMB could therefore improve yields under field conditions. The study observations suggest that there is exploitation potential of SMB in tea production. However, this need confirmation under field conditions in Kenyan.

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